

WHAT IS CLAIMED IS:

- sub B1
- 1 ✓ 1. A method for analyzing a single patient sample to simultaneously
2 determine levels of four biological markers indicative of thyroid disorders, said method
3 comprising:
4 (a) incubating said sample with a mixture of particles in a first suspension,
5 said mixture of particles comprised of groups (i) through (iv):
6 (i) particles coated with anti-thyroid stimulating hormone,
7 (ii) particles coated with anti-triiodothyronine,
8 (iii) particles coated with anti-thyroxine, and
9 (iv) particles coated with a mixture of a diluting agent and a member
10 selected from the group consisting of thyroid peroxidase and anti-
11 human IgG,
12 each group distinguishable from each other group by flow cytometry;
13 (b) recovering said particles from said first suspension, and incubating said
14 recovered particles with a mixture of labeled binding members in a
15 second suspension, said mixture of labeled binding members
16 comprising:
17 (1) labeled anti-thyroid stimulating hormone,
18 (2) a labeled analog composition toward which anti-triiodothyronine
19 and anti-thyroxine have immunological binding affinity, but in
20 which said immunological binding affinity is less than that of
21 anti-triiodothyronine toward triiodothyronine and of anti-
22 thyroxine toward thyroxine, and
23 (3) either labeled anti-human IgG when particles of group (iv) are
24 coated with thyroid peroxidase, or labeled thyroid peroxidase
25 when particles of group (v) are coated with anti-human IgG;
26 said diluting agent being inert toward said biological markers and said labeled
27 binding members; and
28 (c) recovering said particles from said second suspension and detecting the
29 amount of label bound to said particles thus recovered while
30 correlating by flow cytometry the amount of label thus detected to the
31 group to which said label is bound, thereby simultaneously obtaining

32 values individually representative of the levels of thyroid stimulating
33 hormone, triiodothyronine, thyroxine, and anti-thyroid peroxidase.

1 2. A method in accordance with claim 1 in which said particles of
2 group (iv) are coated with a mixture of said diluting agent and anti-human IgG and said
3 labeled binding member (3) is labeled thyroid peroxidase.

1 3. A method in accordance with claim 1 in which:
2 said particles of group (iv) are coated with a mixture of said diluting agent
3 and thyroid peroxidase,
4 said labeled binding member (3) is labeled anti-human IgG,
5 said mixture of particles further comprises group (v), which consists of
6 particles coated with a mixture of a diluting agent and thyroglobulin, and
7 step (c) comprises simultaneously obtaining values individually
8 representative of the levels of thyroid stimulating hormone, triiodothyronine,
9 thyroxine, anti-thyroid peroxidase, and anti-thyroglobulin.

1 4. A method in accordance with claim 1 in which said labeled analog
2 composition of (b)(2) is a single species having immunological binding affinity to both
3 anti-triiodothyronine and anti-thyroxine.

1 5. A method in accordance with claim 4 in which said single species
2 is a member selected from the group consisting of labeled *N-tert*-butyloxycarbonyl-
3 3,5-diiodo-L-thyronine, labeled *N*-acetyl-3-iodo-L-tyrosine, labeled
4 *N-tert*-butyloxycarbonyl-3',3,5-triiodo-L-thyronine, labeled *N-tert*-butyloxycarbonyl-
5 3,5-diiodo-L-tyrosine, labeled *N*-acetylphenylalanyl-3,5-diiodo-L-tyrosine, labeled
6 *N*-acetyl-3,5-dibromo-L-tyrosine, and labeled *N*-acetyl-3,5-diiodo-L-tyrosine.

1 6. A method in accordance with claim 4 in which said single species
2 is labeled *N*-acetyl-3-iodo-L-tyrosine.

1 7. A method in accordance with claim 1 in which said labeled binding
2 members are binding members labeled with fluorescent labels.

1 8. A method in accordance with claim 7 in which said fluorescent
2 labels are B-phycoerythrin.

1 9. A method in accordance with claim 1 in which said labeled analog
2 composition of (b)(2) is a combination of two distinct species, one having immunological
3 binding affinity to anti-triiodothyronine and another having immunological binding
4 affinity to anti-thyroxine.

1 10. A method in accordance with claim 1 in which said labeled binding
2 members are labeled with a common label.

1 11. A method in accordance with claim 1 in which said particles
2 comprise magnetically responsive material and recovery of said particles in steps (b) and
3 (c) is achieved by subjecting said first and second suspensions, respectively, to a
4 magnetic field to cause said particles to adhere to a reaction vessel wall.

1 12. A method in accordance with claim 1 in which said particles
2 incorporate dyes, each of groups (i) through (iv) incorporating a distinct dye that is
3 distinguishable by flow cytometry over the dyes of each other group, and step (c)
4 comprises distinguishing such dyes by flow cytometry while detecting the amount of
5 label bound to said particles.

1 13. A method in accordance with claim 1 in which said diluting agent
2 is a member selected from the group consisting of bovine serum albumin, hydrolyzed
3 porcine gelatin, keyhole limpet hemocyanin, amine-derivatized dextran, and polyacrylic
4 acid.

1 14. A method in accordance with claim 1 in which said diluting agent
2 is bovine serum albumin.

1 15. A method in accordance with claim 1 in which said second
2 suspension of step (b) comprises said recovered particles and said labeled binding
3 members suspended in a buffer solution in which bovine gamma globulin is a solute in a
4 saline solution at approximately physiological pH.

1 16. A method in accordance with claim 1 in which said second
2 suspension of step (b) comprises said recovered particles and said labeled binding
3 members suspended in a buffer solution in which polyethylene glycol is a solute at a
4 concentration of from about 0.5% to about 4.0% by weight.

1 17. A method in accordance with claim 1 in which said second
2 suspension of step (b) comprises said recovered particles and said labeled binding
3 members suspended in a buffer solution in which polyethylene glycol is a solute at a
4 concentration of from about 2.0% to about 3.0% by weight.

1 18. A method in accordance with claim 1 in which said particles of
2 group (iv) have a thyroid peroxidase coating density of from about 0.3ng/cm² to about
3 1.0µg/cm².

1 19. A method in accordance with claim 1 in which said particles of
2 group (iv) have a thyroid peroxidase coating density of from about 0.5ng/cm² to about
3 50ng/cm².

1 20. A method in accordance with claim 1 in which group (i) is
2 comprised of two subgroups differing from each other by particle size such that one
3 subgroup provides a substantially greater sensitivity and is thereby useful for measuring
4 lower concentrations of TSH, than the other.

1 21. A method in accordance with claim 1 in which group (i) is
2 comprised of two subgroups differing from each other by coating density of anti-thyroid
3 stimulating hormone such that one subgroup provides a substantially greater sensitivity
4 and is thereby useful for measuring lower concentrations of TSH, than the other.

1 22. A method in accordance with claim 1 in which group (i) is
2 comprised of two subgroups differing from each other by both particle size and coating
3 density of anti-thyroid stimulating hormone such that one subgroup provides a
4 substantially greater sensitivity and is thereby useful for measuring lower concentrations
5 of TSH, than the other.

1 ✓ 23. A method for analyzing a single patient sample to simultaneously
2 determine levels of thyroid stimulating hormone and anti-thyroxine, said method
3 comprising:
4 (a) incubating said sample with a mixture of particles in a first suspension,
5 said mixture of particles comprised of groups (i) and (ii):
6 (i) particles coated with anti-thyroid stimulating hormone, and

- 7 (ii) particles coated with anti-thyroxine,
8 the groups distinguishable from each other by flow cytometry;
9 (b) recovering said particles from said first suspension, and incubating said
10 recovered particles with a mixture of labeled binding members in a
11 second suspension, said mixture of labeled binding members
12 comprising:
13 (1) labeled anti-thyroid stimulating hormone, and
14 (2) a labeled analog toward which anti-thyroxine has
15 immunological binding affinity, but in which said
16 immunological binding affinity is less than that of anti-
17 thyroxine toward thyroxine; and
18 (c) recovering said particles from said second suspension and detecting the
19 amount of label bound to said particles thus recovered while
20 correlating by flow cytometry the amount of label thus detected to the
21 group to which said label is bound, thereby simultaneously obtaining
22 values individually representative of the levels of thyroid stimulating
23 hormone and thyroxine.

1 24. A method in accordance with claim 23 in which said second
2 suspension of step (b) comprises said recovered particles and said labeled binding
3 member suspended in a buffer solution in which polyethylene glycol is a solute at a
4 concentration of from about 0.5% to about 4.0% by weight.

1 25. A method in accordance with claim 23 in which said second
2 suspension of step (b) comprises said recovered particles and said labeled binding
3 member suspended in a buffer solution in which polyethylene glycol is a solute at a
4 concentration of from about 2.0% to about 3.0% by weight.

add
B4